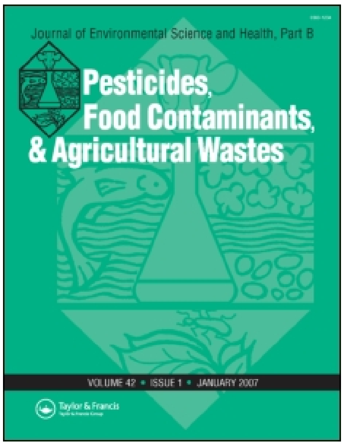

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Propylene Glycol Vapor Contamination in Controlled Environment Growth Chambers: Toxicity to Corn and Soybean Plants

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A major, often unrecognized variable regulating plant growth in semi-closed environment is air contaminant. The vapor of propylene glycol (PG), which was used as coolant in growth chambers, has been found to be toxic to corn (*Zea mays* L.) and soybean (*Glycine max* L.) plants. PG solution leaked from a valve packing system and volatilized to vapor, which was trapped in a semi-closed growth chamber. Symptoms of leaf edge chlorosis, later developing into necrosis, were observed on the third day of emergence or on the third day after moving healthy plants into the chamber. For young soybean plants, symptoms were slightly different from those observed in corn plants; the chlorosis symptoms were not uniformly distributed on all leaves. Some soybean leaves curled up and others had white spots. This problem was identified by using a portable photoionization detector to obtain instantaneous readings of total volatile organic compound concentrations inside the chambers. The presence of PG in selected chambers was verified using sample collection with solid phase microextraction (SPME) followed by analysis with multi-dimensional gas chromatography mass spectrometry (MD-GC-MS). This information is pertinent to researchers who use controlled environment to grow plants.

Key Words: Propylene glycol; Growth chamber; Controlled environment; Air contamination.

INTRODUCTION

Propylene glycol (PG) and ethylene glycol (EG) are commonly used as coolants and deicing solutions for cars, airplanes, and boats. They are also used in

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equipment associated with controlled environment facilities (growth chambers) to regulate temperature inside the chambers. PG is generally regarded as a safe chemical for food use as an additive. However, exposure to EG can cause health problems for humans and animals. Therefore, PG is often used as a less toxic replacement for EG in coolant systems.

Tibbitts[1] and Knight[2] reviewed information on known sources, specific symptoms, and specific compounds of air contaminants in controlled environments that had been identified as air toxicants. However, PG and EG were not listed as known toxic compounds by them. Tibbitts and Peterson[3] reported that EG was toxic to cucumber (*Cucumis sativus* L.) plants with similar symptoms as we observed in corn plants. A number of plants might be injured by PG, where PG leaking was found from an under-bench heating system in a greenhouse. Tibbitts and Peterson[3] therefore suggested that EG, possibly PG, compounds should be added to the list of contaminants in controlled environment.

Laboratory studies (soil-phase) on toxicity of EG, PG, and two formulated glycol aircraft deicing/anti-icing fluids (ADAFs) to lettuce (*Lactuca sativa* L.), perennial ryegrass (*Lolium perenne* L.), a green alga (*Selenastrum capricornutum*), and duckweed (*Lemna minor* L.) were conducted.[4] Test solutions at various concentrations (maximum concentrations were 150, 75, 50, and 49 $g{\cdot}L^{-1}$ for EG, EG ADAF, PG, and PG ADAF, respectively) were added to the culture dishes where test species were grown. While both ADAFs and pure glycols (EG and PG) were toxic to the test species (both root and shoot growth were negatively affected), there were substantial differences in how the species responded to the test solutions. There is a general consensus that chemicals in ADAFs other than glycols are probably responsible for much of the higher toxicity to some species, but these other chemicals have not been identified.[4] This report finds that even low gas-phase concentrations of PG ($<$10 ppm) can cause chlorosis and necrosis in corn and soybean plants.

MATERIALS AND METHODS

Description of Growth Chambers

Twelve sunlit SPAR (Soil-Plant-Atmosphere-Research) growth chambers located at the Henry A. Wallace Beltsville Agricultural Research Center in Beltsville, Maryland, were used for growing corn plants under controlled temperatures and CO_2 concentrations (Fig. 1). The SPAR chambers consist of transparent chamber tops, 2.2 m × 1.4 m × 2.5 m (length × width × height, A) made of 0.0127 m thick Plexiglas G. Each SPAR chamber top is mounted on a 1-m^3 steel soil bin (B). An air handler (C) is attached to the north side of each growth chamber. Pure PG was used as coolant and the coolant valve packing system (Invensys Building Systems, YBA-622-1 and YBA-635, Lovers Park, IL) was

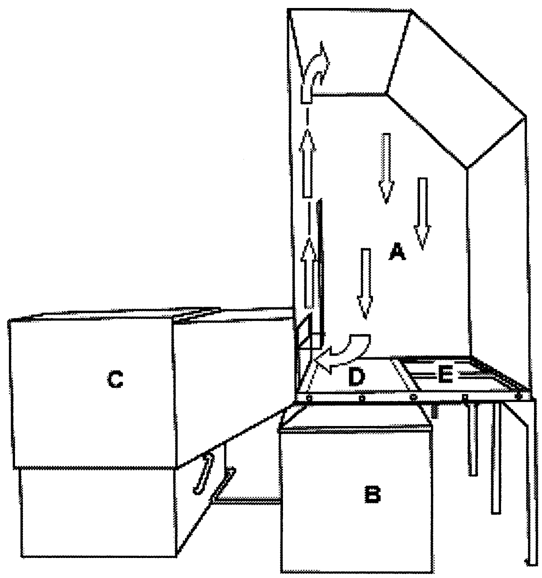

Figure 1: Diagram of soil plant and atmospheric research (SPAR) chamber. Air flow within cuvette and return to air handler is indicated by arrows. *A*: clear Plexiglas cuvette; *B*: one cubic meter soil bin; *C*: air handler containing cooling coils, valves to modulate coolant flow, heaters, and fan. Air is forced into Plexiglas baffle, which directs flow upward; *D*: top of soil bin opens into cuvette; *E*: two gas tight hatches open downward and provide easy access to developing plants.

located inside the air handler. Temperature-controlled air (at 31°C) was recirculated between the handler and the chamber top. The chambers were airtight with an air exchange rate ranging from 0.25 to 0.5 h^{-1}. The constant cycling of the coolant system to maintain constant temperature in the chamber caused wear in the valve packing system and eventually coolant leaks occurred in some chambers. Vapors from the leaking PG were trapped in the chambers and were able to build up to a concentration (>300 ppb) high enough to injure the plants in the chambers.

Verification and Analysis of PG Vapor

In order to determine if there is air contamination inside the chambers, which is responsible for injuring plants (see symptom description below), a portable photoionization detector (PID) (ppbRAE, Rae Systems, Inc., Sunnyvale, CA) was used to obtain an instantaneous measurement of total volatile organic compounds (VOCs) in all 12 chambers. The PID lamp was factory calibrated using isobutylene gas in a range of 0.01–9.99 ppm. PG was sufficiently ioniziable to be detected with the PID, but this detector would also respond to other VOCs present in the chamber air. Air was sampled through a port on the side of each sealed chamber, and the detector was allowed to stabilize over

1–2 min before recording the concentrations. The concentration values should only be considered in relative terms since different chemicals have varying response factors with the PID.

The presence of PG in selected chambers was verified using sample collection with solid phase microextraction (SPME) followed by analysis with multi-dimensional gas chromatography mass spectrometry (MD-GC-MS). A carboxen-polydimethylsiloxane fiber (Sigma-Aldrich Supelco, Bellefonte, PA) with a 75 μm thickness was used as a passive air sampler within five of the chambers. Two chambers had duplicate SPME fibers (average was presented) and one SPME fiber was placed in outside air near the chambers as control.

Mass spectrometry was carried out in electron impact mode with selected-ion monitoring mode to increase sensitivity. Ions specific for PG were monitored (43, 45 m/z). The GC-MS method was developed using a headspace vial containing PG and 30 s exposures of the SPME fiber to the headspace prior to analysis. The system was not calibrated to provide PG air concentrations, but the peak area counts observed by the mass spectrometer can provide a relative comparison of air concentrations between the chambers. The presence of PG in the sample was verified using both a very narrow retention time window, $\pm$0.02 min, and by comparing the ion ratios with a standard.

RESULTS AND DISCUSSION

Plant Symptoms

Symptoms were noticed on the third day after seedlings emerged from the soil surface. Initial symptoms were chlorosis followed by necrosis. The necrotic area expanded with time. The presence of PG in the air did not influence germination. All of the chambers had 100% germination rate at the same time. Symptoms were observed in 8 chambers out of the 12, corresponding to those chambers with moderate to significant PG leakage. Symptoms for newly developed leaves were less severe compared to old leaves, which is the opposite in the case of calcium deficiency in rapidly growing young lettuce leaves.

In another event, healthy corn plants with seven to eight fully expanded leaves were moved into the SPAR chambers. On the third day, similar symptoms of edge necrosis observed equally on all leaves of the plants where PG leak was found in the air handling units. As the exposure time increased, the necrotic area increased. In chambers that had the most severe PG leak, only a small portion of green area was left after two months.

To test if soybean plants would be injured by PG vapor, young, healthy seedlings of soybean were moved into the SPAR chambers with PG leak in the air handler. After five days, injuries were observed. Symptoms were not equally

Table 1: Results of SPME testing for propylene glycol.

Chamber number	Peak area	VOC readings	Symptom of plants
1		3000	Medium
2		450	Minor
3		420	Minor
4	20,831	0	None
5		Out of scale	Minor
6		Out of scale	Minor
7	254,956,544	Out of scale	Severe
8	13,428	0	None
9	30,603,205	Out of scale	Minor
10		Out of scale	Severe
11		Out of scale	Severe
12	56,547,651	Out of scale	Severe
Outside	2,868	0	

seen on all leaves; the necrotic spot/area was not distributed uniformly on all edges. Some leaves had edge burn, some had white spots, and others were curled up.

PG Vapor and VOC Concentrations

The VOC readings in seven chambers with PG leaks exceeded the calibration range of the instrument. Plants in these chambers had more severe symptoms (necrosis areas were larger than 50% of the leaf area by the end of experiment after two months) compared to those where VOC readings were in the range of 300 to 600 ppb (Table 1). Plants exposed to the lower VOC concentrations had minor symptoms with edge necrosis area of less than 25% of the leaf. VOC concentrations in the ambient air outside the chamber were 0 ppb.

Duplicate sample results from the chamber with the largest leak averaged 255 ± 9 million area counts (Table 1, chamber 7). Two other chambers with moderate leaks resulted in 30–56 million area counts. Chambers with no leaks and no plant effects displayed peak areas of 13,000 to 20,000, and the outside control was essentially blank at 2800.

The symptom and VOC readings or peak area were generally in agreement. In chambers 5, 6, and 12 (Table 1), plants had minor symptoms and were developed shortly before VOC measurement was taken. This is because PG leaking from those chambers did not happen from the beginning. Instead, PG leaking occurred at the later part of the experiment and thus the injury symptoms of plants were not severe, although VOC concentration was out of scale.

CONCLUSION

Propylene glycol vapor is toxic to corn and soybean plants. Future studies may be required to determine the threshold-effect air concentrations. Coolant valve packing systems in a controlled environmental facility should be installed in an isolated box where no recirculation with plant growth space will occur. Filtering the air using activated charcoal will alleviate the problem, but it is difficult to completely remove the toxicant in a semi-closed chamber. It is often too late to rescue plants when symptoms are observed.

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